# Anti-CD3 W1

**AAGGACAAGGCCACATTGACTACAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCC** TGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGA CTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAAGTGGAGGTTCTGGT GGAAGTGGAGGTTCAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTG CATCTCCAGGGGAGAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACTG GTACCAGCAGAAGTCAGGCACCTCCCCAAAAGATGGATTTATGACACATCCAAAGTGGCTTCT GATATCAAACTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCT GCAAGACTTCTGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAAAACAGAGGCCTGGACA GGGTCTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTC GGAGTCCCTTATCGCTTCAGTGGCAGTGGGTCTGGGACCTCATACTCTCTCACAATCAGCAGCA TGGAGGCTGAAGATGCTGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCCGCTCACGTTCGG TGCTGGGACCAAGCTGGAGCTGAAA

# **AA Sequence**

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNOKF KDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSG GSGGSGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVAS GVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK

# Fig. 2 A

VH2

GYTNYAQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWG DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSR QGTTVTVSS

## VH3

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSR GYTNYAQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCARYYDDHYCLDYWG QGTTVTVSS

# VH5

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSR GYTNYADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWG QGTTVTVSS

## VH7

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSR GYTNYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYCLDYWG QGTTVTVSS

# Fig. 2 A (cont.)

## ゴゴ×

SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG DIOMTOSPSSLSASVGDRVTITCRASOSVSYMNWYQQKPGKAPKRWIYDT TKVEIK

#### C よ

SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG DIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQOKPGKAPKRWIYDT TKVEIK

## $\Sigma \Sigma$

SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG DIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPGKAPKRWIYDT TKVEIK

# Fig. 2 I

VH2

CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCACAGAAGTTGCAGGGC CGCGTCACAATGACTACAGACACTTCCACCAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT SAGGACACTGCAACCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC SACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAAACCTGGGGCCTCAGTGAAGGTGTCCTGC CAAGGCACCACGGTCACCGTCTCCTCA

### 743

CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCACAGAAGTTGCAGGGC CGCGTCACAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAAATGAACAGCCTGAAAACT SAGGACACTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC SACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACCTGGGGCCTCAGTGAAGGTGTCTGC

### VH5

SACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAAACCTGGGGCCTCAGTGAAGGTGTCTTGC CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCAGACAGCGTCAAGGGC CGCTTCACAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT SAGGACACTGCAACCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGG CAAGGCACCACGGTCACCGTCTCCTCA

# Fig. 2 B (cont.)

# VH7

CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGAC GAGGACACTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACCTGGGGCCTCAGTGAAGGTGTCCTGC AAGGCTTCTGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAGGCCAGGCACCTGGACAGGGT CGCGTCACAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT CAAGGCACCACGGTCACCGTCTCCTCA

# Fig. 2 B (cont.)

#### YI1

SACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCATCTGTCGGGGACCGTGTCACCATCACC GGGACCGACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA <u> AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTCT</u> CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

# VL2

recagaeccaetcaaaetetaaettacateaacteetaccaecaeaaecceeecaaae <u> AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCT</u> GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTCTCCAGGGGAGCGTGCCACCCTGAGC GGGACCGACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA CAGIGGAGIAGIAACCCGCICACGIICGGIGGCGGGACCAAGGIGGAGAICAAA

# VI.3

recagasccastrcaastraastracatsaactsstaccascasaasccssccasaa AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCT GGGACCGACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTGGAGATCAAA 7 / 40

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rig.	4	

#### vH CDR1

Wt anti-CD3 VH2,3 VH5,7 GYTFTRYTMH GYTATRYTMH GYTFTRYTMH

#### vH CDR2

WT anti-CD3, VH7 VH5

YINPSRGYTNYNQKFKD YINPSRGYTNYADSVKG YINPSRGYTNYAQKLQG

#### vH CDR3

VH2, 3

WT anti-CD3, VH2, 3, 5, 7

YYDDHYCLDY

#### vK CDR1

WT anti-CD3, VL3 VL1, 2

RASSSVSYMN RASQSVSYMN

#### vK CDR2

WT anti-CD3, VL1, 2, 3

**DTSKVAS** 

#### vK CDR3

WT anti-CD3, VL1, 2, 3

**QQWSSNPLT** 

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Fig. 2 D

vH CDR1

WT anti-CD3 GGCTACACCTTTACTAGGTACACGATG

CAC

VH2,3 GGCTACACCGCTACTAGGTACACGATG

CAC

VH5,7 GGCTACACCTTTACTAGGTACACGATG

CAC

vH CDR2

WT anti-CD3,

VH7 TACATTAATCCTAGCCGTGGTTATACT

AATTACAATCAGAAGTTCAAGGAC

VH5 TACATTAATCCTAGCCGTGGTTATACT

AATTACGCAGACAGCGTCAAGGGC

VH2,3 TACATTAATCCTAGCCGTGGTTATACT

AATTACGCACAGAAGTTGCAGGGC

VH CDR3

WT anti-CD3,

VH2, 3,

VH5, 7 TATTATGATGATCATTACTGCCTT

GACTAC

### Fig. 2 D (cont.)

#### vK CDR1

WT anti-CD3,

VL3 AGAGCCAGTTCAAGTGTAAGTTACATG

**AAC** 

VL1, 2 AGAGCCAGTCAAAGTGTAAGTTACATG

**AAC** 

vK CDR2

WT anti-CD3,

VL1-3 ACACATCCAAAGTGGCTTCT

VK CDR3

WT anti-CD3,

VL1-3 CAACAGTGGAGTAGTAACCCGCTCACG

#### A) anti-CD3 (VH2/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA CAATGACTACAGACACTTCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

#### B) anti-CD3 (VH2/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAP GQGLEWIGYINPSRGYTNYAQKLQGRVTMTTDTSTSTAYME LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGGADDIQMTQSPSSLSASVGDRVTITCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

#### C) anti-CD3 (VH2/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-CACTTCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT GTCTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGT-CAAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-CAAGGTGGAGATCAAA

#### D) anti-CD3 (VH2/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCOOWSSNPLTFGGGTKVEIK

#### E) anti-CD3 (VH2/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-CACTTCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT GTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-CAAGGTGGAGATCAAA

#### F) anti-CD3 (VH2/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

#### A) anti-CD3 (VH3/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA CAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAA ATGAACAGCCTGAAAACTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

#### B) anti-CD3 (VH3/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCARYYDD-HYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIQMTQSPSSLSASVGDRVTITCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

#### C) anti-CD3 (VH3/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA CAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAA ATGAACAGCCTGAAAACTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

#### D) anti-CD3 (VH3/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAP GQGLEWIGYINPSRGYTNYAQKLQGRVTMTTDTSTSTAYLQ MNSLKTEDTAVYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

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# Figure 4 E) anti-CD3 (VH3/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-CACTTCCACCAGCACAGCCTACCTGCAAATGAACAGCCT-GAAAACTGAGGACACTGCAGTCTATTACTGTGCAAGATATT ATGATGATCATTACTGCCTTGACTACTGGGGCCCAAGGCAC-CACGGTCACCGTCTCCTCAGGCGAAGGTACTAG-TACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGAC-GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTC TCCAGGGGAGCGTGCCACCCTGACCTGCAGAGCCAGTT-CAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGG-CAAGGCACCCAAAAGATGGATTTATGACACATCCA-AAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGG TCTGGGACCGACTACTCTCTCACAATCAACAGCTTG-GAGGCTGAAGATGCTGCCACTTATTACTGCCAACAGTG-GAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTG-GAGATCAAA

#### F) anti-CD3 (VH3/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

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# Figure 5 A) CD3 (VH5/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCAGACAGCGTCAAGGGCCGCTTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

#### B) CD3 (VH5/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAP GQGLEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYME LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGSGGADDIQMTQSPSSLSASVGDRVTITCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

#### C) anti-CD3 (VH5/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCAGACAGCGTCAAGGGCCGCTTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

#### D) anti-CD3 (VH5/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAP GQGLEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYME LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

#### E) anti-CD3 (VH5/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCTTTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACG-CAGACAGCGTCAAGGGCCGCTTCACAATCACTACAGACA-AATCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAG-CAGACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCT CTGTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGC-CAACAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGG-GACCAAGGTGGAGATCAAA

#### F) anti-CD3 (VH5/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-ADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

#### A) anti-CD3 (VH7/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACAATCAGAAGTTCAAGGACCGCGTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

#### B) anti-CD3 (VH7/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYT-NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIQMTQSPSSLSASVGDRVTITCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCOOWSSNPLTFGGGTKVEIK

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#### Figure 6

#### C) anti-CD3 (VH7/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACAATCAGAAGTTCAAGGACCGCGTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

#### D) anti-CD3 (VH7/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYT-NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQOWSSNPLTFGGGTKVEIK

#### E) anti-CD3 (VH7/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCTTTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACAAT-CAGAAGTTCAAGGACCGCGTCACAATCACTACAGACA-AATCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT GTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-CAAGGTGGAGATCAAA

#### F) anti-CD3 (VH7/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYT-NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

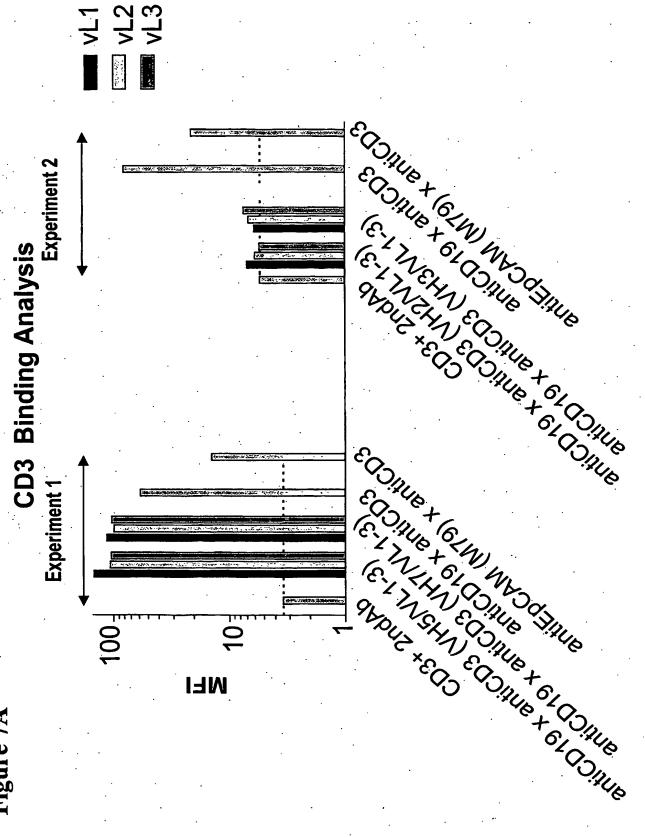
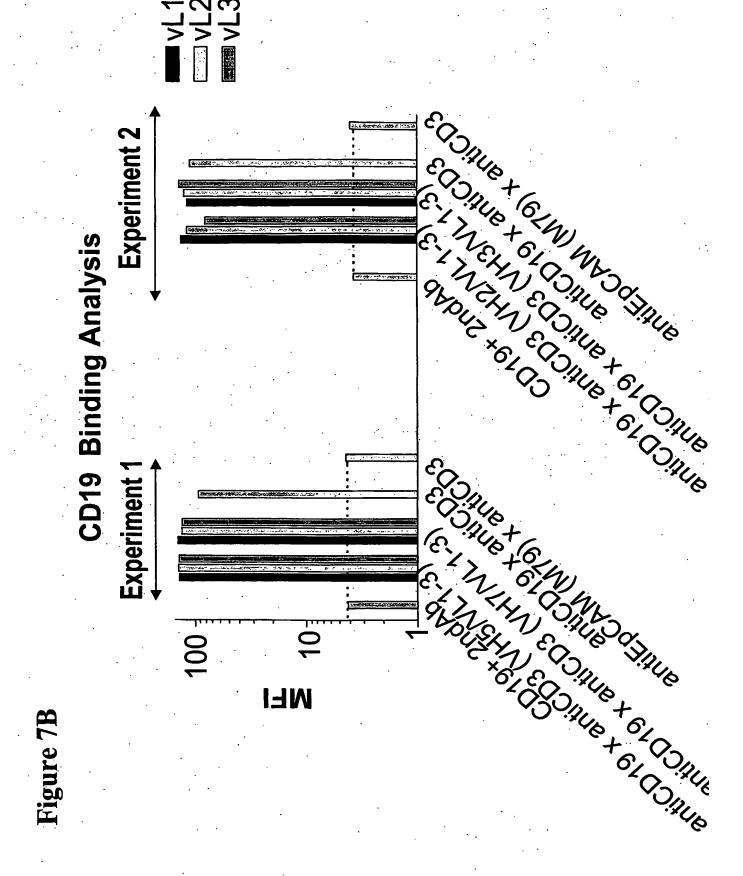


Figure 7.





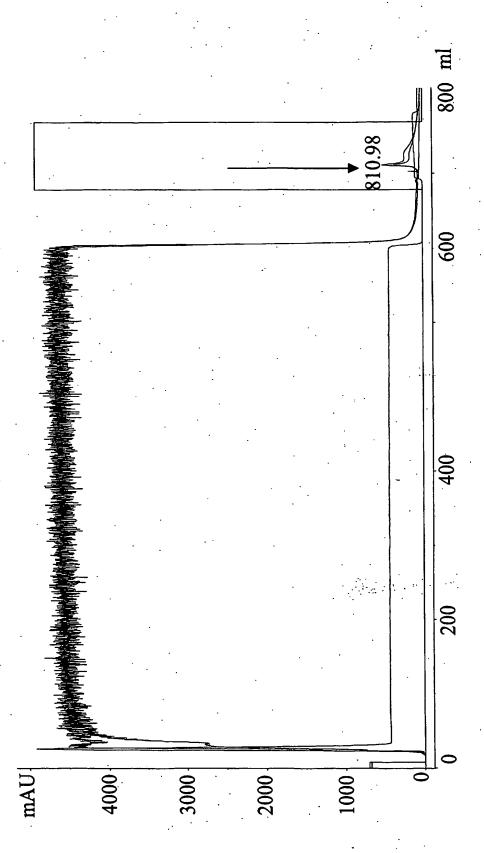


Figure 9

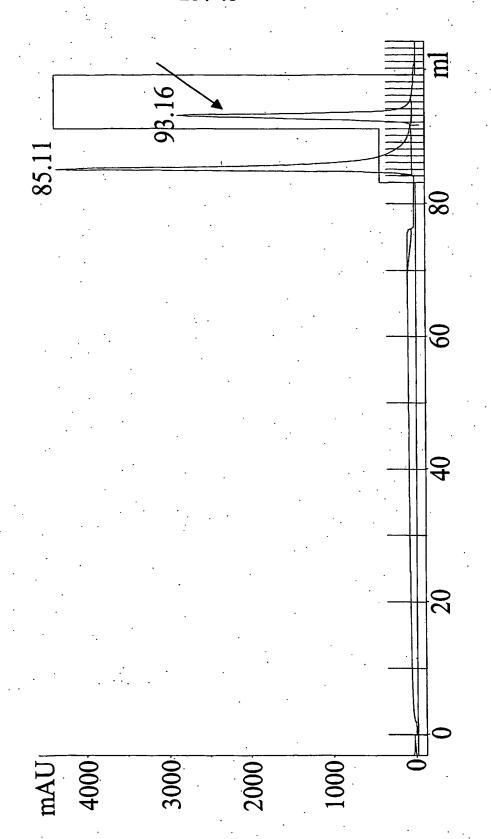


Figure 10

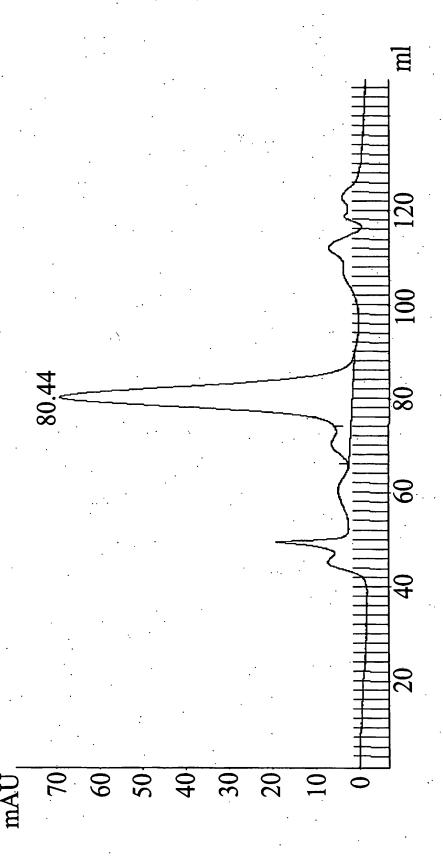


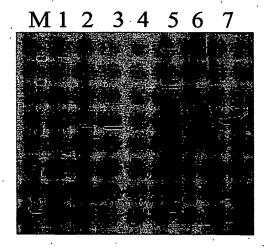
Figure 11

**A)** 

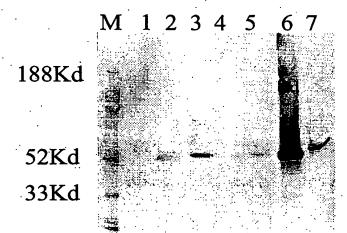
188KD

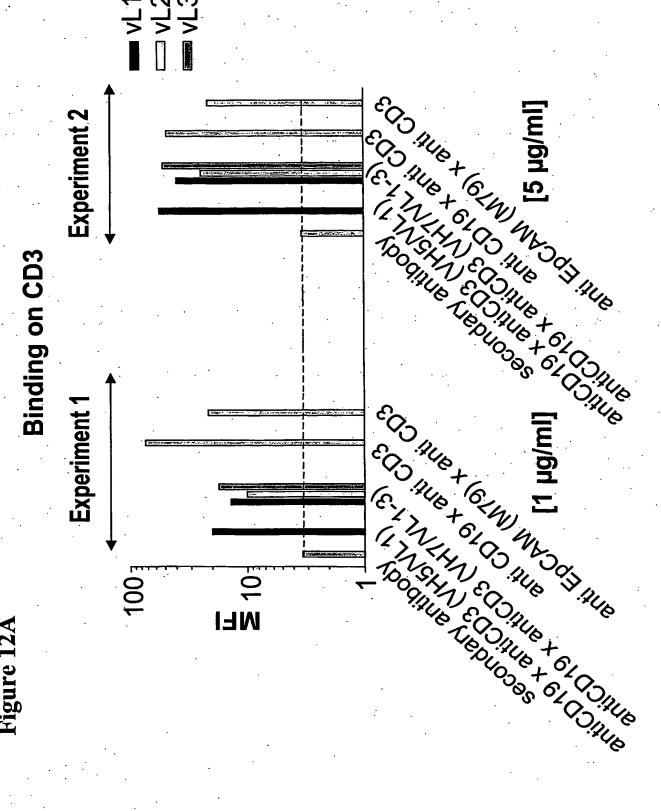
52 Kd

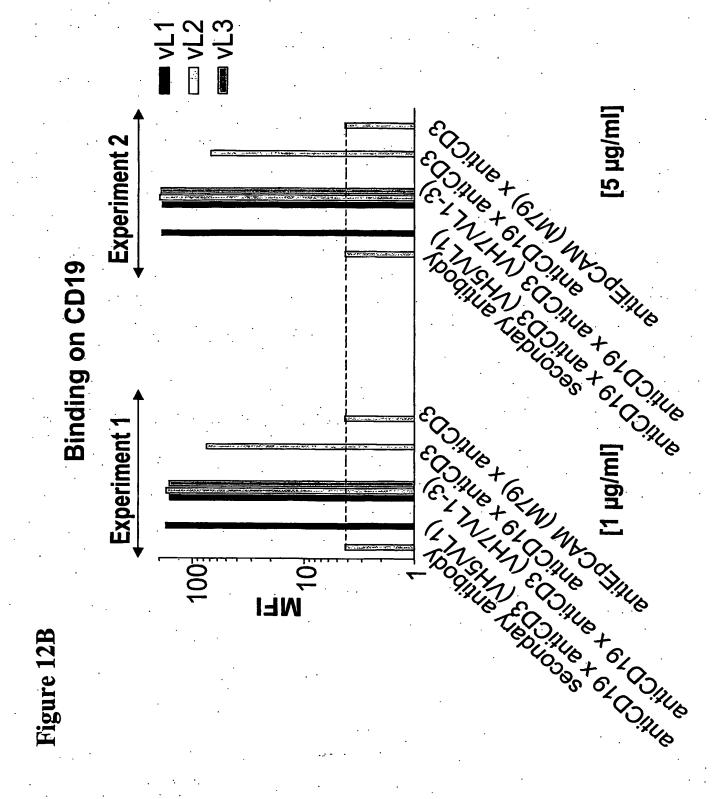
33Kd

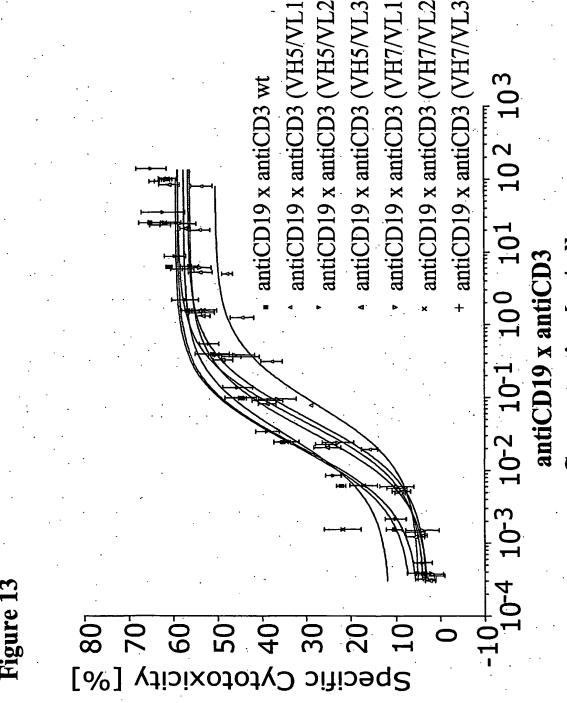


**B**)





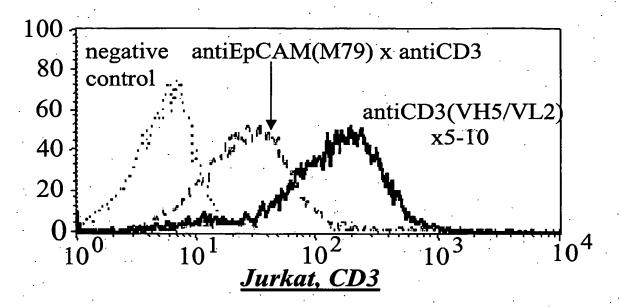




•		FRI	CDR1	FR2	CDR2
nondeimmunized	ğ				-
anti-CD3	DIKLQQSGAELA	RPGASVKMSCKTS	YTETRYTMH	WVKQRPGQGLEWIGY	DIKLOOSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNOKFKD
anti-CD3 VH5	DVQLVQSGAEVK	KPGASVKVSCKAS	SYTETRYTMH	WVRQAPGQGLEWIGY	DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSRGYTNYADSVKG
anti-CD3 VH7	DVQLVQSGAEVK	KPGASVKVSCKASC	<b>YTETRYTMH</b>	WVRQAPGQGLEWIGY	DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSRGYTNYNQKFKD
anti-co3 VH2	DVQLVQSGAEVK	KPGASVKVSCKASC	SYTATRYTMH	WVRQAPGQGLEWIGY	DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSRGYTNYAQKLQG
anti-CD3 VH3	DVQLVQSGAEVK	KPGASVKVSCKAS	YTATRYTMH	WVRQAPGQGLEWIGY	DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSRGYTNYAQKLQG
			٠.	٠	. ,

RVTMTTDTBTSTAYLQMNSLKTEDTAVYYCARYYDDHYCLDYWGQGTTVTVSS RETRITIOK BISTAN MELS BIRSED TATYYCARYYDDHYCLD YMGOGITVTVSS RVTLTTDKFTSTAYMELSBLRSEDTAVYYCARYYDDHYCLDYWGQGTTVTVSS RVTMTTDTSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSS **SSSTAYMQLSBLTSEDSAVYYCAR\YYDDHYCLDYWGQGTTLTVSS** FR3 KATHITOK nondeimmunized anti-CD3 VH5 anti-CD3 VH3 anti-CD3 VH7 anti-co3 VH2 anti-CD3

Figure 15 A antiCD3(VH5/VL2) x 5-10 (SEQ ID NO: 37)



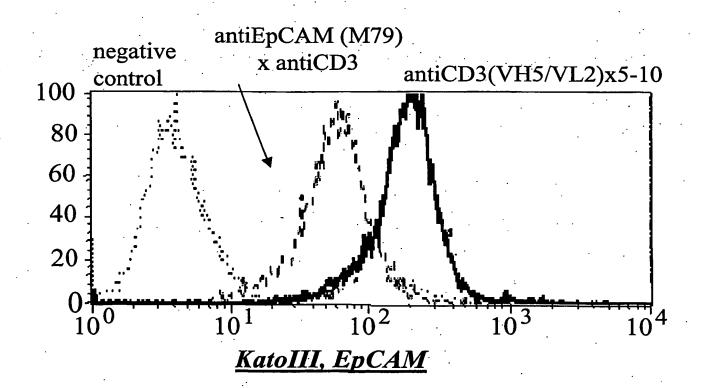
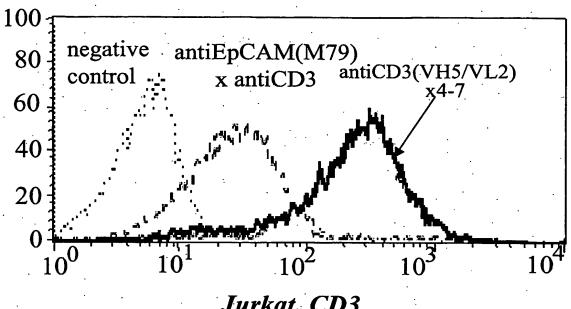


Figure 15B

#### antiCD3(VH5/VL2) x 4-7 (SEQ ID NO:33)



### Jurkat, CD3

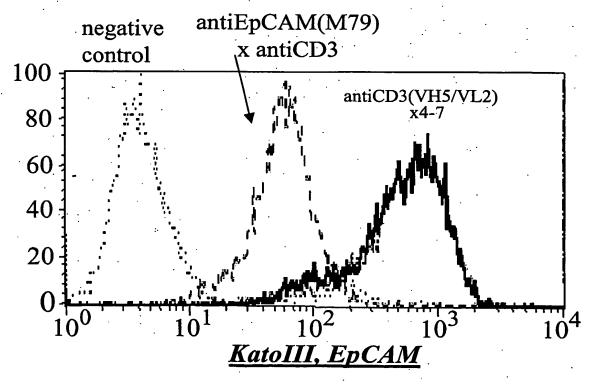
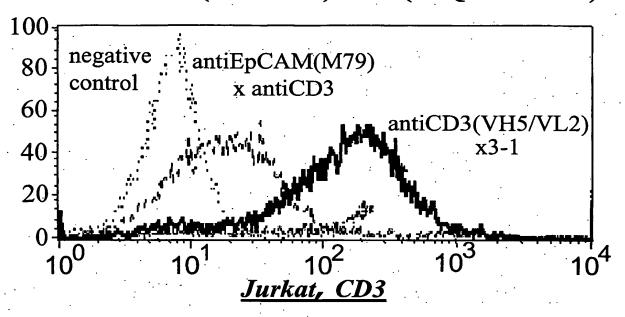


Figure 15C

#### antiCD3(VH5/VL2) x 3-1 (SEQ ID NO:31)



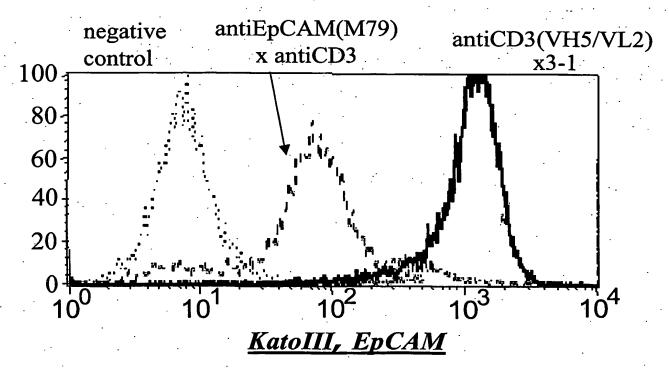
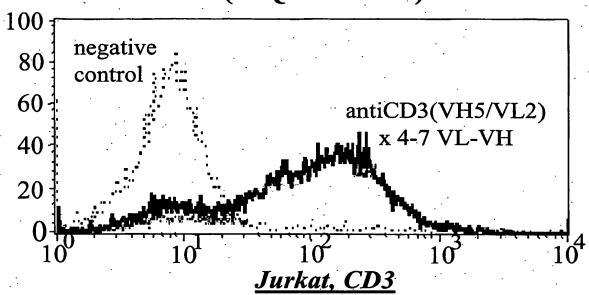
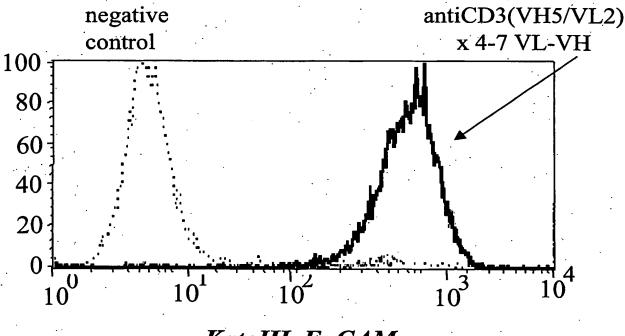


Figure 15 D

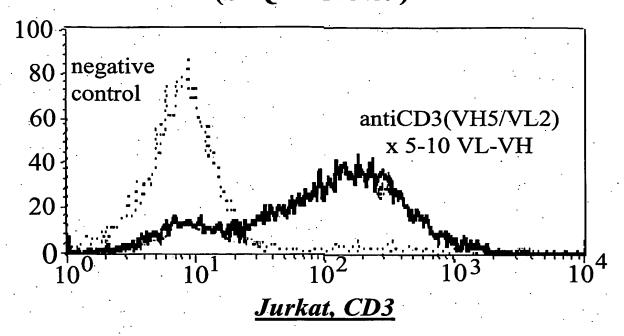






KatoIII, EpCAM

Figure 15 E antiCD3(VH5/VL2) x 5-10 VL-VH (SEQ ID NO:39)



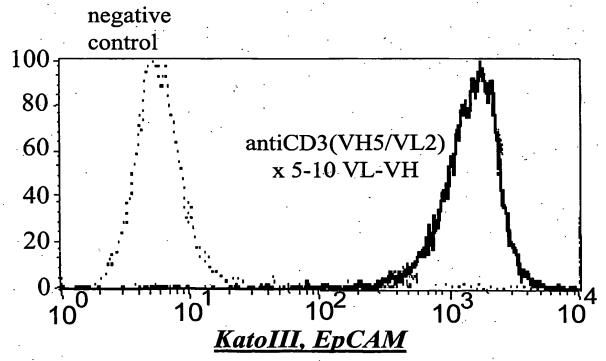
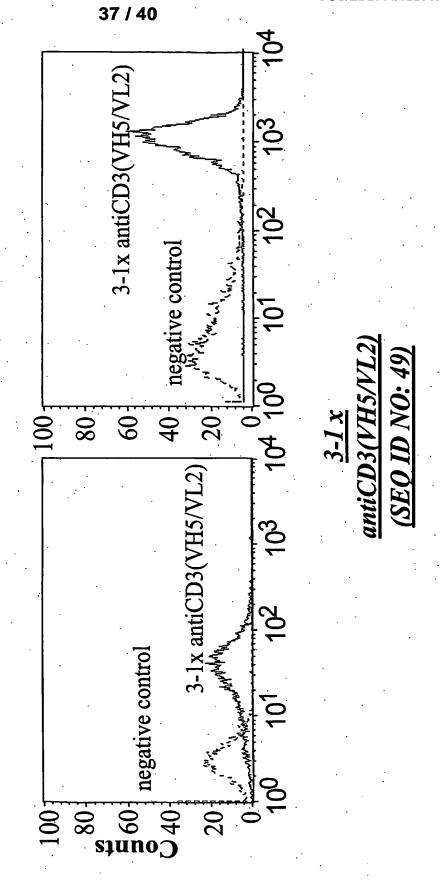


Figure 16 A

CD3 Binding (Jurkat



EpCAM Binding (Kato cells)

CD3 Binding (Jurkat cells)

Figure 16 B

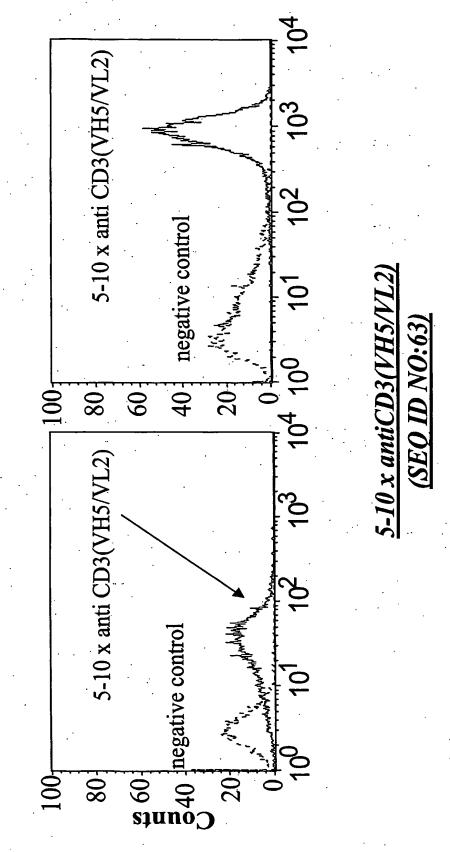
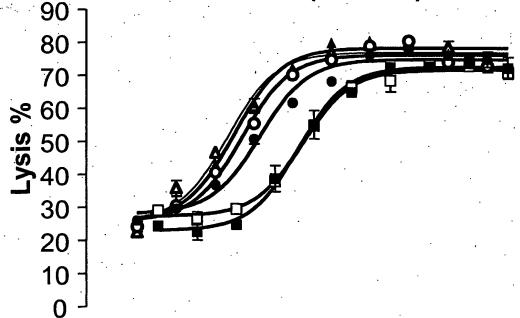


Figure 17

- wt antiCD3 x 3-1
- □ di antiCD3(VH5/VL2) x 3-1
- wt antiCD3 x 5-10
- O di antiCD3 (VH5/VL2)  $\times$  5-10
- ▲ wt antiCD3 x 4-7
- $\Delta$  di antiCD3(VH5/VL2) x 4-7



10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup> 10<sup>6</sup> 10<sup>7</sup> bispecific construct [pg/ml]

Figure 18

- 3-1 x antiCD3
- □ 3-1 x antiCD3(VH5/VL2)
- 5-10 x antiCD3
- o 5-10 x antiCD3(VH5/VL2)

